

Determination of Polyphenols in Beer by an Effective Method Based on Solid-Phase Extraction and High Performance Liquid Chromatography with Diode-Array Detection

MARKÉTA DVOŘÁKOVÁ, PETR HULÍN, MARCEL KARABÍN and PAVEL DOSTÁLEK

Department of Fermentation Chemistry and Bioengineering, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

DVOŘÁKOVÁ M., HULÍN P., KARABÍN M., DOSTÁLEK P. (2007): **Determination of polyphenols in beer by an effective method based on solid-phase extraction and high performance liquid chromatography with diode-array detection.** Czech J. Food Sci., 25: 182–188.

The determination of polyphenols by spectrophotometric detection is complicated due to their low concentrations in beer. The beer samples have to be pre-concentrated before using the spectrophotometric detection for their quantification. An analytical method based on solid-phase extraction (SPE) and followed by high performance liquid chromatographic separation with diode-array detection is used for the determination of free gallic, protocatechuic, caffeic, *p*-coumaric, ferulic and salicylic acids, of (+)-catechin, (–)-epicatechin, and quercetin. These phenolic compounds participate in colloidal and sensory stability of beer. Six different SPE cartridges were tested and three different types of elution with the most appropriate solvents (acetonitrile, acetone and methanol) were used. The performance of the HPLC method was assessed by the evaluation of parameters such as absolute recovery, relative standard deviation (RSD – lower than 10%), the limit of quantification (LOQ), and the limit of detection (LOD). The polyphenol content in various types of Czech beer is presented.

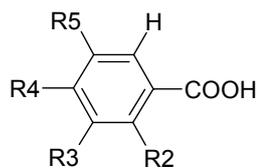
Keywords: polyphenols; beer; HPLC; SPE; spectrophotometric detection

Phenolic compounds are secondary metabolites naturally present in plants. They have great importance for the food and drink products derived from plants, since these compounds are responsible for their organoleptic properties. As a consequence, they are closely related to the quality of such products which makes their analysis considerably interesting as described by ROBBINS (2003).

The beer contains a complex mixture of phenolic compounds extracted from malt and hops which

have been shown to have useful antioxidant properties as described by GOUPY *et al.* (1999). Moreover, three groups of polyphenols are responsible for beer flavour and physical stability as described by a number of authors (SHAHIDI & NACZK 1995; GOUPY *et al.* 1999; ČEPIČKA & KARABÍN 2002). Simple polyphenols derived from hydroxybenzoic acids (gallic acid, protocatechuic acid, etc.) and hydroxycinnamic acids (ferulic acid, *p*-coumaric acid, caffeic acid, etc.) are extracted mostly from

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Centre 1M0570).

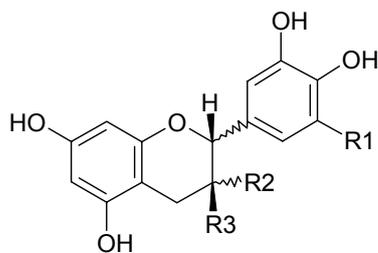


R ₂	R ₃	R ₄	R ₅	Name of the acid
H	H	H	H	benzoic
OH	H	H	H	salicylic
H	OH	OH	H	protocatechuic
OH	H	H	OH	gentistic
H	OH	OH	OH	gallic

Figure 1. Phenolic acids derived from benzoic acid

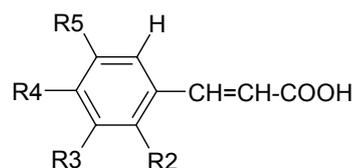
malt but are also present in small amounts in hops. Their structures are shown in Figures 1 and 2. Flavonols (quercetin, kaempferol etc.) come mostly from hop. Flavan-3-ols, including monomers such as (+)-catechin and (–)-epicatechin (Figure 3), dimers (prodelphinidin B3 and procyanidin B3), trimers (procyanidin C2), flavonoid-derived tannins up to higher molecular weights, arise equally from malt and hop. The final content of phenolic components of beer depends on both the raw materials and the brewing process.

For quality control, it is necessary to evaluate phenolic compounds by rapid analytical method because they can affect beer flavour and stability. Analytical methods for determining phenolic compounds in wort and beer are limited. Several authors determined phenolic compounds in beer matrices by direct injection HPLC after filtration, as described by ANDERSEN *et al.* (2003). Another method is HPLC separation and online detection by diode-array spectroscopy after a chemical reaction with *p*-dimethylaminocinnamaldehyde (DMACA) as described by PASCUAL-TERESA *et al.* (2000). Nowadays, the solid-phase extraction (SPE) is



(+)-catechin (R₁ = H, R₂ = H, R₃ = OH)
 (–)-epicatechin (R₁ = H, R₂ = OH, R₃ = H)

Figure 3. Structure of monomeric flavan-3-ols



R ₂	R ₃	R ₄	R ₅	Name of the acid
H	H	H	H	cinnamic
H	H	OH	H	<i>p</i> -coumaric
H	OCH ₃	OH	H	ferulic
H	OH	OH	H	caffeic

Figure 2. Phenolic acids derived from cinnamic acid

the common technique used for pre-concentration and purification prior to HPLC separation of phenolic compounds in wines, described by a number of authors (BETES-SAURA *et al.* 1996; GUILLEN *et al.* 1997; KARAGIANNIS *et al.* 2000). The most common method of the determination of polyphenols in wines, olive oils and other foods and drinks is HPLC with mass spectrometric detection described by ROBBINS (2003). The separation of phenolic compounds in beer was performed commonly by reversed liquid chromatography followed by ultraviolet detection described by HAYES *et al.* (1987), photodiode-array detection described by a number of authors (SÁNCHEZ *et al.* 1988; ES-SAFI *et al.* 1999; MONTANARI *et al.* 1999), fluorimetric detection described by DVOŘÁKOVÁ and DOSTÁLEK (2006), electrochemical detection described by SKERIKOVA *et al.* (2004) or mass spectrometric detection described by WHITTLE and ELDRIDGE (1999).

MATERIAL AND METHODS

Reagents. Standards of gallic acid (97% purity) CAS No. [149-91-7], protocatechuic acid (97% purity) CAS No. [99-50-3], caffeic acid (99% purity) CAS No. [331-39-5], gentistic acid (98% purity) CAS No. [490-79-9], *p*-coumaric acid (98% purity) CAS No. [501-98-4], salicylic acid (99% purity) CAS No. [69-72-7], ferulic acid (99% purity) CAS No. [1135-24-6], (+)-catechin hydrate (98% purity) CAS No. [88191-48-4], (–)-epicatechin (90% purity) CAS No. [490-46-0] and quercetin dihydrate (98% purity) CAS No. [6151-25-3] were purchased from SigmaAldrich (Germany). Further used were solvents such as methanol super gradient from LabScan (Ireland), acetonitrile for HPLC from

SigmaAldrich (Germany), and acetone from Penta (Czech Republic).

A stock standard solution (ca. 500 mg/l) of each phenolic compound was prepared in methanol by weighing out approximately 0.025 g of the analyte into 50 ml volumetric flask and diluting to the respective volume. The mixed standard solution was prepared by dilution the mixed stock standard solutions in methanol to give a concentration of ca. 50 mg/l for each polyphenol. All standard solutions were stored in the dark at 5°C and were stable for at least three months.

Samples. The seven different types of Czech beer (labelled sample 1–7) produced by different manufacturers in the Czech Republic were tested and purchased at local markets. Four types of lager beer (samples 2, 3, 4, 7), one type of draught beer (sample 1), and two non-alcoholic beers (sample 5, 6) were analysed. The samples were stored in the dark at low temperature (5°C).

The unstabilised lager beer (sample 7) was used for testing SPE cartridges with the content of alcohol 4.8 vol. % and with the addition of 1 ml mixed standard solution (spiked beer solution).

Solid-phase extraction. 25 ml of the sample was submitted to the SPE process. This extraction is performed in a vacuum device SPE Vacuum Manifold Dorcus of Tessek (Prague, Czech Republic) using 6 different kinds of cartridges. We used Waters Oasis[®] Max cartridge (Milford, USA) of 60 mg of sorbent with reversed-phase and anion-exchange functionalities (encapped with quarternary amine groups), AccuBond II ODS-C18 (Agilent, USA) of 500 mg of sorbent with reversed-phase functionality (endcapped silica with octadecyl groups), Discovery DSC-Ph (Supelco, Germany) of 500 mg of monomerically bonded phenyl, Discovery DSC-C8 (Supelco, Germany) of 500 mg of sorbent with reversed-phase functionality (modified silica with octyl groups), Discovery DPA-6S (Supelco, Germany) of 500 mg of sorbent with reversed-phase functionality (modified silica with amid groups), Supelclean LC-18 (Supelco, Germany) of 500 mg of sorbent with reversed-phase functionality (modified silica with octadecyl groups). The cartridges were selected due to their affinity to the analytes. The detailed characterisation of the sorbents used for SPE is described by DVOŘÁKOVÁ and DOSTÁLEK (2006).

The cartridge was conditioned with 5 ml of methanol followed by 10 ml of distilled water. An aliquot part of the beer sample (25 ml) or spiked

beer solution, previously acidified to pH 1.5 with hydrochloric acid 36%, was passed through the cartridge. Subsequently, phenolic compounds were eluted with 12 ml of acetonitrile (in the case of DPA-6S, 12 ml of acetone was used for the elution, and in the case of Oasis[®] Max, we used 12 ml of methanol for the elution according to the application lists). The organic eluate was transferred into a 50 ml round-bottomed flask and evaporated under vacuum at 35°C to dryness. The residue obtained was dissolved in 1 ml of mobile phase (methanol:water 50:50, acidified with 1% acetic acid) and transferred to a vial. Certain samples had to be filtered through a cellulose filter (Millipore) 0.45 µm before the transfer to the vial.

Determination of total polyphenols (EBC). According to the EBC method 9.11, we measured the content of total polyphenols. This method is described in detail in ANONYMOUS (1998). The determination of total polyphenols in beer by spectrophotometry.

The 10 ml of degassed beer sample and 8 ml of CMC/EDTA reagent (Carboxymethyl cellulose/ethylenediaminetetraacetic acid) were transferred to a 25 ml volumetric flask and thoroughly mixed the content. Then 0.5 ml of ferric reagent (3.5% ammonium iron citrate) was added to the sample, which was then thoroughly homogenised. After that, 0.5 ml of ammonia reagent (ammonia:water, 1:2) was added and thoroughly mixed. Finally, the volume was made up to 25 ml with distilled water and homogenised. The absorbance at 600 nm was measured after 10 min, for reaction to take place and stabilise.

To obtain the content of polyphenols, the following formula was used:

$$P = A \times 820$$

where: P – polyphenol content (mg/l)

A – absorbance at 600 nm

Chromatographic system and operating conditions. The analysis was performed with a HPLC system Alliance 2695 separation module with Photo Diode Array detector 2996 connected to a PC computer running the software program Empower (Waters, Milford, USA).

The analytical column (150 × 3.9 mm) used was a Waters Nova-Pak 4 µm C₁₈ (Milford, USA) with guard column (5 × 3.9 mm) Waters Nova-Pak 4 µm C₁₈. For HPLC analysis, an aliquot (10 µl) was injected onto the column and eluted at the temperature of 20°C. The gradient conditions were in accordance with GARCÍA *et al.* (2004).

Table 1. Absolute recovery (%) of phenolic substances \pm standard deviation (%) (sample 7)

Compound	Oasis [®] -MAX	AccuBond C18	DPA-6S	DSC-C8	DSC-Ph	LC-C18
Gallic acid	9.1 \pm 0.9	14.3 \pm 2.0	34.5 \pm 2.1	24.3 \pm 1.3	21.5 \pm 1.3	22.9 \pm 3.2
Protocatechuic acid	67.3 \pm 0.2	95.1 \pm 0.3	67.1 \pm 4.8	92.7 \pm 9.6	11.1 \pm 1.3	14.6 \pm 4.0
Catechin	87.5 \pm 1.9	93.0 \pm 16.3	56.4 \pm 3.6	78.6 \pm 5.8	103.5 \pm 2.7	74.4 \pm 22.6
Caffeic acid	63.7 \pm 2.4	15.2 \pm 4.7	56.0 \pm 2.5	31.1 \pm 2.0	14.8 \pm 1.8	25.8 \pm 2.1
Epicatechin	65.6 \pm 0.1	15.4 \pm 2.8	40.7 \pm 4.7	64.1 \pm 8.1	17.0 \pm 0.7	37.8 \pm 11.1
<i>p</i> -Coumaric acid	72.6 \pm 0.7	34.7 \pm 5.0	85.5 \pm 4.2	93.3 \pm 4.2	34.6 \pm 1.6	70.6 \pm 9.1
Ferulic acid	63.8 \pm 1.8	72.4 \pm 6.4	102.5 \pm 8.4	92.6 \pm 15.8	92.5 \pm 2.8	62.4 \pm 18.5
Salicylic acid	77.8 \pm 4.1	78.1 \pm 0.5	63.2 \pm 0.1	106.7 \pm 19.8	84.6 \pm 0.3	102.0 \pm 50.4
Quercetin	102.7 \pm 1.6	76.7 \pm 1.0	96.2 \pm 5.7	111.8 \pm 14.4	47.0 \pm 6.0	75.5 \pm 8.0

The mobile phase was acidified to ensure the total protonation of the compounds studied. For UV detection, the wavelength program was optimised to monitor phenolic compounds at their respective maximum absorbance wavelengths as follows: λ 280 nm held for 12.7 min, changed to λ 330 nm and held for 2 min, changed to λ 306 nm and held for 2.9 min, changed to λ 280 nm and held for 3.1 min, changed to λ 306 nm and held for 15.3 min, changed to λ 280 nm and held for the rest of the analysis.

The detection and quantification of quercetin, gallic acid, protocatechuic acid, (+)-catechin, and (–)-epicatechin was done at 280 nm, of caffeic acid, *p*-coumaric acid, and salicylic acid at 306 nm, and of gentistic acid at 330 nm, respectively.

RESULTS AND DISCUSSION

Solid-phase extraction – optimisation of the method

For the extraction and purification of polyphenols, the removal of interfering compounds and recovery, 6 kinds of SPE cartridges were tested. Commercial beer samples (25 ml) were previously degassed and acidified to pH 1.5, spiked with 1 ml of mixed standard solution (the concentration of polyphenols being 50 mg/l) at the concentration of the added polyphenols of 2 mg/l. After equilibration, the spiked beer samples were processed according to the procedure described. Acetonitrile, methanol (Oasis[®]-MAX) and acetone (DPA-6S) were used for the elution of the analytes from SPE cartridges (12 ml) according to their application lists. Triplicate analyses were performed with each cartridge. The absolute re-

covery was calculated as the percentage content of polyphenols in the samples after SPE as related to the concentration of polyphenols in the spiked sample before SPE. The results are presented in Table 1. The ability in removing of interfering compounds is demonstrated in Figures 4 and 5. These cartridges show good recoveries and also low standard deviations for the compounds determined. Other cartridges with very high recoveries (presented in Table 1), DSC-C8 and LC-C18, unfortunately revealed very high standard deviations for several compounds determined. It shows that DSC-C8 and LC-C18 do not have a good repeatability and should not be used.

Method performance

The quality parameters such as recovery values, relative standard deviation (RSD), the limits of

Table 2. Method performance: relative standard deviation, limit of detection and limit of quantification (sample 7, cartridge Oasis[®]-MAX)

Compound	RSD (%)	LOD (mg/l)	LOQ (mg/l)
Gallic acid	8	0.05	0.15
Protocatechuic acid	2	0.11	0.38
(+)-Catechin	4	0.29	0.78
Caffeic acid	3	0.06	0.21
(–)-Epicatechin	4	0.23	0.76
<i>p</i> -Coumaric acid	2	0.04	0.14
Ferulic acid	2	0.07	0.22
Salicylic acid	5	0.41	0.80
Quercetin	1	0.16	0.54

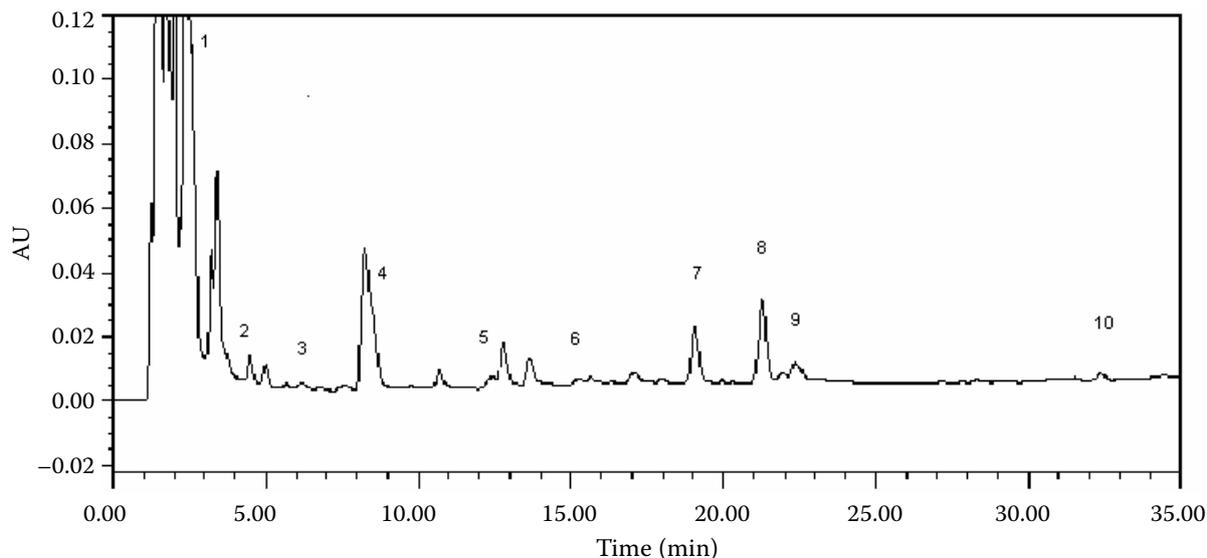


Figure 4. Chromatogram of spiked beer sample before SPE: 1 – gallic acid, 2 – protocatechuic acid, 3 – gentistic acid, 4 – catechin, 5 – caffeic acid, 6 – epicatechin, 7 – *p*-coumaric acid, 8 – ferulic acid, 9 – salicylic acid, 10 – quercetin

detection (LOD) and quantification (LOQ), were evaluated with the cartridge Oasis[®]-MAX. The method performance results are demonstrated in Table 2. The recovery and RSD of the method were assessed by analysing 5 samples ($n = 5$) on the same day. The values show good precision of the method proposed (RSD < 10%, recovery > 60% except gallic acid).

The limits of detection (LOD) and quantification (LOQ) were evaluated on the basis of the signal obtained ($n = 5$). LOD and LOQ were defined as the

concentration of the analyte that produced the signal to noise ratio of three and ten, respectively; lower than 0.4 and 0.8 mg/l for all target compounds.

Polyphenol content in beer samples

Seven beer samples produced by different Czech breweries were analysed. The identification of the peaks was carried out by their retention times in comparison with the standards, and the UV spectra of the samples and standards acquired by

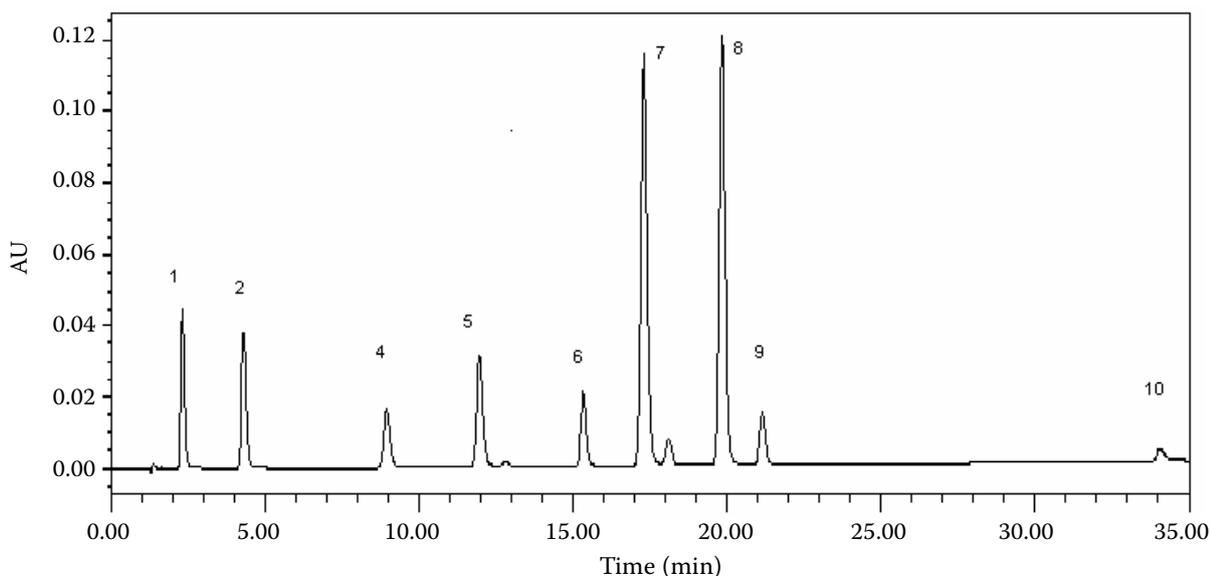


Figure 5. Chromatogram of spiked beer sample after SPE on DPA-6S cartridge: 1 – gallic acid, 2 – protocatechuic acid, 3 – gentistic acid, 4 – catechin, 5 – caffeic acid, 6 – epicatechin, 7 – *p*-coumaric acid, 8 – ferulic acid, 9 – salicylic acid, 10 – quercetin

Table 3. Polyphenol content (mean value \pm standard deviation in mg/l) in beer samples with SPE on Oasis[®]-MAX, ND < LOD

Oasis [®] -MAX c (mg/l)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Total polyphenol content	125	130	127	128	91	70	242
Gallic acid	0.28 \pm 0.02	0.42 \pm 0.01	ND	0.28 \pm 0.02	0.47 \pm 0.03	0.39 \pm 0.03	0.29 \pm 0.02
Protocatechuic acid	0.45 \pm 0.04	0.58 \pm 0.03	0.43 \pm 0.03	0.53 \pm 0.05	0.78 \pm 0.04	0.77 \pm 0.07	0.43 \pm 0.04
Gentistic acid	ND	ND	ND	ND	0.35 \pm 0.05	ND	ND
(+)-Catechin	2.78 \pm 0.09	1.66 \pm 0.10	1.41 \pm 0.04	2.07 \pm 0.09	1.70 \pm 0.11	1.62 \pm 0.11	3.75 \pm 0.13
Caffeic acid	1.50 \pm 0.06	1.34 \pm 0.10	1.19 \pm 0.06	1.44 \pm 0.13	1.38 \pm 0.11	1.48 \pm 0.11	1.38 \pm 0.08
(-)-Epicatechin	1.19 \pm 0.06	1.12 \pm 0.08	0.89 \pm 0.08	1.21 \pm 0.08	1.48 \pm 0.11	1.39 \pm 0.09	1.29 \pm 0.08
<i>p</i> -Coumaric acid	2.08 \pm 0.09	2.22 \pm 0.17	2.18 \pm 0.03	2.12 \pm 0.16	1.82 \pm 0.13	1.72 \pm 0.07	2.55 \pm 0.17
Ferulic acid	2.74 \pm 0.21	4.32 \pm 0.36	3.00 \pm 0.23	3.47 \pm 0.18	2.48 \pm 0.16	2.60 \pm 0.16	5.04 \pm 0.42
Salicylic acid	3.39 \pm 0.30	3.99 \pm 0.29	3.50 \pm 0.20	4.38 \pm 0.07	3.33 \pm 0.04	1.98 \pm 0.03	2.25 \pm 0.16
Quercetin	1.72 \pm 0.16	1.72 \pm 0.10	1.79 \pm 0.10	1.74 \pm 0.05	1.82 \pm 0.07	1.53 \pm 0.07	1.72 \pm 0.05

ND = not determined

photodiode array detector were compared. The results of the polyphenol content determination in the beer samples are shown in Table 3. Total polyphenol content ranged from 70 to 242 mg/l. The content of ferulic acid ranged from 2.48 to 5.04 mg/l, of salicylic acid from 1.98 to 4.38 mg/l, and of *p*-coumaric acid from 1.72 to 2.55 mg/l; these were found to be the highest. On the other hand, the content of gentistic acid ranged from ND to 0.35 mg/l and that of (-)-epicatechin ranged from 0.89 to 1.48 mg/l; these were found in the lowest concentrations. Ferulic acid concentrations are higher than *p*-coumaric acid concentrations since ferulic acid is formed from *p*-coumaric acid via the shikimic acid pathway as described by KENYHERCZ and KISSINGER (1977).

The levels of polyphenols found are in agreement with the data already published. McMURROUGH *et al.* (1984) determined the total content of phenolic acids in beers. The concentration range found was from 5 to 8 mg/l. The predominant acids were vanilic, *p*-coumaric, and ferulic acids. HAYES *et al.* (1987) determined the phenolic compounds commonly as benzoic acid derivatives, protocatechuic and gallic acids as cinnamic acid derivatives, caffeic acid, *p*-coumaric and ferulic, finally (+)-catechin and (-)-epicatechin were also quantified. FLORIDI *et al.* (2003) determined 19 phenolic compounds in beer; average values of 23 different samples were 0.6 mg/l for gallic acid, 0.84 mg/l for protocatechuic acid, 0.4 mg/l for gentistic acid, 0.6 mg/l for caffeic acid, 1.4 mg/l for *p*-coumaric

acid, 2.4 mg/l for ferulic acid, and 2.9 mg/l for salicylic acid, respectively. GARCÍA *et al.* (2004) compared the contents of ten polyphenols in nine alcohol-free beer samples produced in different Spanish breweries. Total content of the polyphenols selected ranged from 3.5 to 8.5 mg/l. The content of ferulic acid ranged from 0.7 to 2.3 mg/l, of *p*-coumaric acid from 0.1 to 0.7 mg/l, of caffeic acid from 0.2 to 0.4 mg/l, of protocatechuic acid from 0.7 to 5.1 mg/l, and finally of (+)-catechin from 0.3 to 4.5 mg/l.

CONCLUSIONS

The optimised method is presented as an analytical tool for the determination of phenolic compounds in beer. The most convenient SPE cartridges for the pre-concentration of polyphenols and elimination of interfering substances that provide a low standard deviation and a good recovery are Oasis[®]-MAX anion-exchange cartridge and polyamide cartridge DPA-6S. Their application to commercial beer samples ensures that the resulting levels of polyphenols do not affect the beer flavour and stability. Also very good recoveries were found with cartridges DSC C8 and LC C18, unfortunately, however, with very high standard deviations for the majority of the compounds determined. Gentistic acid was not detected in most of the samples.

The performance of the method for Oasis[®]-MAX was assessed by the evaluation of parameters such

as absolute recovery (higher than 60%, except of gallic acid), relative standard deviation (lower than 10%), limit of detection (ranged from 0.04 to 0.41 mg/l), and limit of quantification (ranged from 0.14 to 0.80 mg/l).

References

- ANONYMOUS (1998): Analytica EBC (Analytica European Brewery Convention). 5th Ed. Verlag Hans Carl Getränke-Fachverlag, Nürnberg.
- ANDERSEN M.L., LAURIDSEN R.K., SKIBSTED L.H. (2003): Optimizing the use of phenolic compounds in foods, Phytochemical Functional Foods. CRC Press LLC, Boca Raton: 315–346.
- BETES-SAURA C., ANDRÉS-LACUEVA C., LAMUELA-RAVENTOS R.M. (1996): Phenolics in white free run juices and wines from Penedés by High-Performance Liquid Chromatography. *Journal of Agriculture and Food Chemistry*, **44**: 3040–3046.
- ČEPIČKA J., KARABÍN M. (2002): Polyfenolové látky piva – přirozené antioxidanty. *Chemické Listy*, **96**: 90–95.
- DVOŘÁKOVÁ M., DOSTÁLEK P. (2006): Analytical methods for determination of polyphenols in sweet wort, wort and beer. *Kvasný Průmysl*, **52**: 111–114.
- ES-SAFI N., FULCRAND H., CHEYNIER V., MOUTONET M. (1999): Competition between (+)-catechin and (–)-epicatechin in acetaldehyde-induced polymerization of flavonols. *Journal of Agriculture and Food Chemistry*, **47**: 2088–2095.
- FLORIDI S., MONTANARI L., MARCONI O., FANTOZZI P. (2003): Determination of free phenolic acids in wort and beer by coulometric array detection. *Journal of Agriculture and Food Chemistry*, **51**: 1548–1554.
- GARCÍA A.A., GRANDE B.C., GÁDARA J.S. (2004): Development of rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for determination of polyphenols in alcohol-free beers. *Journal of Chromatography, A*, **1054**: 175–180.
- GOUPY P., HUGUES M., BOIVIN P., AMIOT M.J. (1999): Antioxidant compounds of barley (*Hordeum vulgare*) and malt extracts. *Journal of the Science of Food and Agriculture*, **79**: 1625–1634.
- GUILLEN D.A., MERELLO F., BARROSO C.G., PEREZ-BUSTAMANTE J.A. (1997): Solid phase extraction for sample preparation in the HPLC analysis of polyphenolic compounds in “Fino” sherry wine. *Journal of Agriculture and Food Chemistry*, **45**: 403–406.
- HAYES J.P., SMYTH M.R., MCMURROUGH I. (1987): Comparison of electrochemical and ultraviolet detection in High-Performance Liquid Chromatography for the determination of phenolic compounds commonly found in beers. *Analyst*, **112**: 1197–1207.
- KARAGIANNIS S., ECONOMOU A., LANARIDIS P. (2000): Phenolic and volatile composition of wines made from *Vitis vinifera* cv. Muscat lefko grapes from the island of Samos. *Journal of Agriculture and Food Chemistry*, **48**: 5369–5375.
- KENYHERCZ T.M., KISSINGER P.T. (1977): A new approach to the phenolic components in beer. Application to the determination of sinapic, ferulic, and *p*-coumaric acids. *Journal of Agriculture and Food Chemistry*, **25**: 959–961.
- MCMURROUGH I., ROCHE G.P., CLEARY K.G. (1984): Phenolic acid in beers and worts. *Journal of the Institute of Brewing*, **90**: 181–187.
- MONTANARI L., PERRETI G., PATELLA F., GIUDI A., FANTOZZI P. (1999): Organic and phenolic acids in beer. *Lebensmittel-Wissenschaft & Technologie*, **32**: 535–539.
- PASCUAL-TERESA S., SANTOS-BUELGA C., RIVAS-GONZALO J.C. (2000): Quantitative analysis of flavan-3-ols in spanish foodstuffs and beverages. *Journal of Agriculture and Food Chemistry*, **48**: 5331–5337.
- ROBBINS R.J. (2003): Phenolic acids in foods: An overview of analytical methodology. *Journal of Agriculture and Food Chemistry*, **51**: 2866–2887.
- SÁNCHEZ F.G., CARNERO C., HEREDIA A. (1988): Determination of *p*-coumaric and ferulic acids in mixtures by isodifferential derivative spectrophotometry. *Analytical Letters*, **21**: 1243–1257.
- SHAHIDI F., NACZK M. (1995): Food Phenolics; Sources, Chemistry, Effects, Applications. Technomic Publishing Co. Inc., Lancaster: 10–13.
- SKERIKOVA V., GRYNova L., JANDERA P. (2004): Using CoulArray detector for analysis of natural antioxidants. *Chemické Listy*, **98**: 343–348.
- WHITTLE N., ELDRIDGE H. (1999): Identification of the polyphenols in barley and beer by HPLC/MS and HPLC/electrochemical detection. *Journal of the Institute of Brewing*, **105**: 89–99.

Received for publication August 31, 2006
Accepted after corrections April 18, 2007

Corresponding author:

Ing. MARKÉTA DVOŘÁKOVÁ, Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav kvasné chemie a bioinženýrství, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 220 444 036, fax: + 420 220 445 051, e-mail: marketa.dvorakova@vscht.cz